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Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice to Proposed Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its next meeting, the Director of the National Institute of Allergy and Infectious Diseases will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by February 1, 1982.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland, 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules, as well as actions under these Guidelines.

1. Proposed Revision of Guidelines

Dr. Waclaw Szybalski, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, has requested that the following sentence be added to the current NIH Guidelines:

"These are only Guidelines based on the current state of the research and on the concerns of certain individuals or groups about the hypothetical risks. Individual researchers should use their best scientific judgement and peers' advice when designing, modifying and conducting experiments, and be prepared to justify any substantial variations when requested, or in their applications and progress reports."

2. Request To Use Bacillus Megaterium in Recombinant DNA Experiments Under P1 Containment

Dr. Patricia Vary of Northern Illinois University, DeKalb, Illinois, requests permission to introduce recombinant DNA derived from *Staphylococcus aureus, E. coli,* and *Bacillus subtilis* into *Bacillus megaterium* under P1 conditions.

3. Proposed EK2 Host-Vector Systems

Dr. Roy Curtiss, University of Alabama, Birmingham, Alabama, requests EK2 certification of six different *E. coli* K–12 strains in conjunction with various virulent and temperate bacteriophage lambda, plasmid and cosmid vectors. Dr. Curtiss also requests that all previously approved vectors be approved as vector components of the proposed EK2 host-vector systems. Detailed information on the proposed host-vector systems has been provided by Dr. Curtiss.

4. Proposed use of EK2 Host-Vector Systems for Cloning DNA from Class 3 and 4 Etiologic Agents

Dr. Roy Curtiss, University of Alabama, Birmingham, Alabama, requests permission to use all certified EK2 host-vector systems to clone DNA fragments from Class 3 and Class 4 etiologic agents under P2 containment, and under P1 containment if the recombinant clones are shown not to express a virulence determinant that has toxic potential.

As an alternative to this general proposal, Dr. Curtiss requests permission to clone DNA from *Yersinia pestis* and *Mycobacterium leprae* into EK2 host-vector systems under P2 containment, and under P1 conditions in the absence of expression of virulence determinants by the recombinant clones.

5. Proposed Pseudomonas Putida Host-Vector System

Dr. Michael Bagdasarian of the Max-Planck Institute of Fur Molekulare Genetik, Berlin, West Germany, requests HV1 certification of a host-vector system based on *Pseudomonas putida* strain KT2440 and cloning vectors pKT262, pKT263, and pKT264.

6. Proposed Inclusion of Yersinia Enterocolitica on Sublist A, Appendix A

Dr. Guy Cornelis of the Universite Catholique de Louvain, Brussels, Belgium, requests that *Yersinia enterocolitica* be exempted from the Guidelines under Section I–E–4 and added to Sublist A, Appendix A, on the basis that this species exchanges genetic information with *E. coli.*

7. Proposal for Revision of the Guidelines

Dr. Susan Gottesman of the National Cancer Institute of the National Institutes of Health has requested that the following proposal to modify the NIH Guidelines for Research Involving Recombinant DNA Molecules be published in the **Federal Register** for comment:

I. Introduction

There seems to be a clear consensus that the guidelines require simplification, reorganization, and possible lowering of containment levels for some classes of experiments. The Recombinant DNA Advisory Committee (RAC) will be considering at its next meeting a proposal, published for comment in the **Federal Register**, to convert the NIH Recombinant DNA Guidelines into a voluntary code of practice. This proposal would change the detailed listing of containment levels to a few paragraphs of advice to the investigator. I am recommending an alternative approach to revision which retains some aspects of the current guidelines which I consider necessary for the continued orderly development of the recombinant DNA field.

(1) My proposal, as opposed to the RAC proposal, retains the requirement for IBC oversight of some experiments, and retains the current NIH compliance requirements. In addition, the record-keeping function of the IBC is retained. Since many experiments became exempt from the requirements of the Guidelines as of July 1, 1981, the remaining requirements should not be too cumbersome to either the experimenter or the IBC.

(2) My proposal simplifies the structure of the Guidelines and decreases containment for some classes of experiments. In particular, experiments involving non-pathogenic prokaryotes and lower eukaryotes could be carried out at P1 containment. Overall, containment levels would not, however, be lowered to the extent recommended in the RAC proposal.

II. Summary of Proposed Changes as Compared to Current Guidelines

A. *Prohibitions.* The word "prohibition" will no longer be applied to this class of experiments which

currently require explicit RAC review and NIH approval.

1. Three of the five prohibitions (I–D–2, I–D–4, and I–D–5 in the current Guidelines) would be listed in a new section designated III–A and entitled "Experiments that require FAC review and NIH approval before initiation." These three prohibitions cover experiments involving the deliberate formation of recombinant DNAs containing genes for the biosynthesis of certain toxins lethal for vertebrates (I–D–2), the deliberate release into the environment of organisms containing recombinant DNA (I–D–4), and the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture (I–D–5).

2. Prohibition I–D–1 in the current guidelines (formation of recombinant DNAs derived from CDC Class 4 or 5 organisms) would be deleted. Such experiments could be carried out under specific conditions set by the IBC, as described in a new section designated III–B and entitled "Experiments that require IBC approval before initiation".

3. Prohibition I–D–6 in the current Guidelines, which covers certain large-scale experiments, would be eliminated; such experiments could proceed after IBC approval (new section III–B–5). Currently the Guidelines permit certain large scale experiments involving *E. coli* K–12, *S. cerevisiae* and *B. subtilis* host-vector systems to proceed following review by the local IBC (46 FR 53983).

B. *Exemptions.* No changes in the substance of this section are proposed. The categories of exemptions now listed in Section I–E would be listed in a new Section III–D.

C. *Section II (Containment).* All references to HV3 systems would be deleted. Such systems are not presently in use and are not required by current or proposed guidelines. Otherwise, the section would remain as is.

D. *Section III (Containment Guidelines for Covered Experiments).* The complete text of Section III would be replaced by new text which would reorganize, simplify, and eliminate redundancy in the current Guidelines. In addition, significant lowering of containment for some classes of experiments is recommended. In the new Section III, all experiments would fall into one of four classes:

III–A. Current prohibitions; such experiments would require specific RAC review and NIH approval.

III–B. Experiments requiring prior review by the IBC. These would include the use of recombinant DNA molecules derived from or propagated in pathogens, non-defective animal virus systems, and the insertion of recombinant DNA into whole animals and plants. Many experiments which would be included in this section currently require NIH approval.

III–C. Experiments requiring notice to the IBC simultaneously with initiation of the experiment.

The proposal would change the procedure for reviewing recombinant DNA experiments involving non-pathogenic prokaryotes, lower eukaryotes, or defective animal virus systems. These experiments currently require prior review by the IBC. Under this proposal, investigators would not have to wait for IBC approval before initiating these experiments. In addition, containment requirements for experiments involving non-pathogenic prokaryotes and non-pathogenic lower eukaryotes would be relaxed.

III–D. Currently exempt experiments. No changes in the substance of this section are proposed.

E. Section IV. Changes as necessary to reflect changes in other sections of the Guidelines would be incorporated.

F. Much material currently in Appendix E would be covered or superseded by the proposed changes in the Guidelines in Section III. Appendix D would be deleted.

III. Changes in Current Guidelines to Implement Proposal

A. *Current Section I.* Section I–A, *Purpose,* would remain unchanged.

Section I–B, *Definition of Recombinant DNA Molecules,* would be modified to include a statement on synthetic DNAs, and would read as follows:

"I–B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

"Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) shall be considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, it is exempt from the Guidelines."

Section I–C, *General Applicability,* would remain unchanged.

Section I–D, *Prohibitions,* would be deleted. (Some of this material is included in proposed new Section III–A).

Section I–E, *Exemptions,* would be deleted. (This material would be included in proposed new Section III–D).

B. *Current Section II.* Delete current Section II–D–1–c and subsections II–D–1–c–(1) through II–D–1–c–(5), Section II–D–2–b–(3) and references to HV3 in Section II–D–3.

C. *Current Section III.* Delete and replace current Section III and all subsections of III with the following text:

"Part III discusses experiments involving recombinant DNA. These experiments have been divided into four classes:

"III–A. Experiments which require specific RAC review and NIH approval before initiation of the experiment;

"III–B. Experiments which require IBC approval before initiation of the experiment;

"III–C. Experiments which require IBC notification at the time of initiation of the experiment;

"III–D. Experiments which are exempt from the procedures of the Guidelines.

"If an experiment falls into both class III–A and one of the other classes, the rules pertaining to class III–A must be followed. If an experiment falls into class III–D and into either class III–B or III–C as well, it can be considered exempt from the requirements of the Guidelines.

"Changes in containment levels from those specified here may not be instituted without the express approval of the Director, NIH (See Sections IV–E–1–b–(1), IV–E–1–b–(2), and subsections).

"III–A. *Experiments that Require RAC Review and NIH Approval Before Initiation.* Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the **Federal Register** for thirty days of comment, review by the RAC, and specific approval by NIH. The containment conditions for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments also require the approval of the IBC before initiation. Specific experiments already approved in this section and the appropriate containment conditions are listed in Appendices E and G.

"III–A–1. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae*

neurotoxin). Specific approval has been given for the cloning in *E. coli* K–12 of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Containment levels for these experiments are specified in Appendix G.

"III–A–2. Deliberate release into the environment of any organism containing recombinant DNA.

"III–A–3. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquistion could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

"III–B. *Experiments that Require IBC Approval Before Initiation.* Investigators performing experiments in this category must submit to their Institutional Biosafety Committee (IBC), prior to initiation of the experiments, a registration document that contains a description of: (a) The source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene, and, if so, what protein will be produced, and (e) the containment conditions specified in these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Requests for lowering of containment for experiments in this category will be considered by NIH (See Section IV–E–1–b–(3)).

"III–B–1. *Experiments Using CDC Class 2, Class 3, Class 4, or Class 5 Agents* as Host-Vector Systems.*

"III–B–1–a. Experiments involving the introduction of recombinant DNA into CDC Class 2 agents can be carried out at P2 containment.

"III–B–1–b. Experiments involving the introduction of recombinant DNA into CDC Class 3 agents can be carried out at P3 containment.

"III–B–1–c. Experiments involving the introduction of recombinant DNA into CDC Class 4** or Class 5 agents can be carried out at P4 containment. A USDA permit is required for work with Class 5 agents***.

"III–B–2. *Experiments in Which DNA from CDC Class 2 or Class 3 Agents* is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.*

"III–B–2–a. Recombinant DNA experiments in which DNA from CDC Class 2 or Class 3 agents* is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under P2 containment. Specific lowering of containment to P1 for particular experiments can be approved by the IBC. Many experiments in this category will be exempt from the Guidelines (See Sections III–D–4 and III–D–5). Experiments involving the formation of recombinant DNAs for certain toxin genes requires RAC review and NIH approval (see Section III–A–1), or must be carried out under NIH specified conditions as described in Appendix G.

"III–B–2–b. Recombinant DNA experiments in which DNA from CDC Class 4** or Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes can be performed at P2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's viral genome is present in a given recombinant. In the absence of such a demonstration, P4 containment should be used.

Note.—A USDA permit is required for work with Class 5 pathogens***.

"III–B–3. *Experiments Involving the Use of Whole Animal or Plant Viruses or Defective Animal or Plant Viruses in the Presence of Helper Virus in Tissue Culture Systems.*

"III–B–3–a. Experiments involving the use of whole CDC Class 2 animal viruses*, or defective CDC Class 2 animal viruses* in the presence of helper virus, can be performed at P2 containment.

"III–B–3–b. Experiments involving the use of whole CDC Class 3 animal viruses*, or defective CDC Class 3 animal viruses* in the presence of helper virus, can be carried out at P3 containment.

"III–B–3–c. Experiments involving the use of whole CDC Class 4 or Class 5 animal viruses*, or defective CDC Class 4 or Class 5 animal viruses* in the presence of helper virus, may be carried out under P4 containment. A USDA permit is required for work with CDC Class 5 pathogens***.

"III–B–3–d. Experiments involving the use of whole animal or plant viruses, or defective animal or plant viruses in the presence of helper virus, not covered by Sections III–B–3–a, III–B–3–b, or III–B–3–c may be carried out under P1 containment.

"III–B–4. *Recombinant DNA Experiments Involving Whole Animals and Plants.*

"III–B–4–a. DNA from any source except for greater than one quarter of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (2A).

"III–B–4–b. For all experiments involving whole animals and plants and not covered by III–B–4–a, the appropriate containment will be determined by the IBC.

"III–B–5. *Experiments Involving More than 10 Liters of Culture.*

The appropriate containment will be decided by the IBC. Where appropriate, the large scale containment recommendations of the NIH should be used (45 FR 24968).

"III–C. *Experiments that Require IBC Notice Simultaneously with Initiation of Experiments.*

"For experiments in this category, a registration document as described in Section III–B must be dated and signed by the investigator and filed with the local IBC. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. All experiments not included in categories III–A, III–B, III–D, and subsections of these categories are to be considered as in category III–C. All such experiments can be carried out at P1 containment. Some explicit cases are listed below.

"III–C–1. *Experiments Involving Non-Pathogenic Prokaryotes or Non-Pathogenic Lower Eukaryotic Host-Vector Systems.*

"Experiments involving non-pathogenic prokaryotes or non-pathogenic lower eukaryotes can be carried out at P1 containment.

"III–C–2. *Experiments Involving Formation of Recombinant DNA Molecules Containing no More Than Two-Thirds of the Genome of any Eukaryotic Virus.*

"Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (36) being considered identical (50)) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

"III–D. *Exempt Experiments.*

"The following recombinant DNA molecules are exempt from these Guidelines and no registration with the IBC is necessary.

"III–D–1. Those that are not in organisms or viruses.

"III–D–2. Those that consist entirely of DNA segments from a single non-

chromosomal or viral DNA source, though one or more of the segments may be synthetic equivalent.

"III–D–3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

"III–D–4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

"III–D–5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland 20205."

D. *Current Section IV. Roles and Responsibilities.* Changes as necessary to reflect changes in Parts I–III only.

E. *Current Section V. Footnotes and References.* Changes as necessary to reflect changes in Parts I–III.

F. *Current Appendices.* Delete sections subsumed by proposed guideline changes; i.e. delete Appendix D and entries 1, 2, 3, 5, 7, 10, 11, 12, 14, 15, 17, 18, 20, 22, 24, 25, 26, 27, 28, 29, 33, and 34 of Appendix E.

Footnotes

* *Classification of Etiologic Agents on the basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

** All activities, including storage of variola and whitepox are retricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

*** A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service USDA, Federal Building. Hyattsville, MD 20782.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require serveral additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

Dated: November 25, 1981.

Richard M. Krause,

Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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